REMARKS

The Office Action of August 7, 2002 presents the examination of claims 1-31. Claims 1, 3-5, 10-11, 14, 16, 23, 26-27, and 30-31 are amended. Claim 32 is added. Support for claim 32 is found in the last two line of page 9 of the specification. Claims 12, 21, 22, 24, and 25 are canceled. No new matter is inserted into the application.

Drawings

Applicants note that the drawings were approved by the Draftsperson, as noted in the Interview Summary dated August 21, 2002.

Oath/Declaration

The Examiner requires the submission of a new oath or declaration that acknowledges the filing of foreign applications. Applicants submit herewith under separate cover a new Declaration, which acknowledges the filing of PCT/IN00/00058 filed in India on May 30, 2000.

Claim Objections

The Examiner objects to claims 4, 10, 16, and 23-24 for various minor errors. Claim 24 is canceled, thus rejection the objection thereto moot. In response to the Examiner's remarks,

Applicants amend claims 4, 16, and 23 in accordance with the Examiner's suggestions. Thus, the instant objection applied to these claims is overcome.

However, Applicants do not agree with the Examiner that the recitation of "and" in line 3 of claim 10 should be replaced with "acid." In fact, the Examiner's suggestion is incorrect. Throughout the Office Action, the Examiner refers to "*Arabidopsis* acid *Acetolactate synthase* gene". Applicants advise the Examiner that *Arabidopsis* and *Acetolactate synthase* are two different things. *Acetolactate synthase* is a gene and *Arabidopsis* is a plant. *Acetolactate synthase* is obtained from *Arabidopsis*. There is no such thing as "*Arabidopsis* acid…." As such, Applicants do not amend claim 10 and the objection thereof should be withdrawn.

The Examiner also objects to claims 12, 25, and 26 for allegedly being in improper dependent form. Claims 12 and 25 are canceled, thus rendering the objection applied to these claims moot. Claim 26 is specifically objected to for failing to refer to claims in alternative form. In response to the Examiner's remarks, Applicants amend claim 26 to be dependent from claim 16 only. Thus, the instant objection is overcome.

Rejection under 35 U.S.C. § 112, first paragraph

Enablement

The Examiner rejects claims 1-25 and 27-31 under 35 U.S.C. § 112, first paragraph, for allegedly containing subject matter not

enabled by the specification. Claims 12, 21, 22, 24, and 25 are canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Specifically, the Examiner maintains her position that the specification, while being enabling of insulator sequences that *do not* comprise enhancers or other types of transcriptional regulatory elements, does not provide enablement for insulator sequences that *do* comprise enhancers or other types of transcriptional regulatory elements.

In the Reply filed on April 10, 2002, Applicants argued against the rejection and presented new claim 31, which recites that the insulator sequence does not encode any functional or regulatory components or possess any regulatory or enhancer elements or sequences that may influence the expression of neighboring genes. Applicants insert the subject matter of claim 31 into the independent claims 1 and 16 herein. Specifically, claims 1 and 16 are amended to recite that the insulator sequence does not comprise transcriptional or other regulatory or enhancer elements. Applicants respectfully point out to the Examiner that the claims should not be rejected, since the Examiner has acquiesced that insulator sequences that *do not* comprise enhancers or other types of transcriptional regulatory elements are enabled by the specification.

Furthermore, Applicants point out that the present invention uses a sequence having the properties set out in claims 10 and 11 as an insulator sequence. Any DNA sequence that contains enhancers or other transcriptional or other regulatory elements cannot be used as an insulator sequence. Example 1 is provided to illustrate the teachings of the present invention (see pages 18-19 of the specification). Example 1 uses *topoisomerase* from pea and *acetolactate synthase* from *Arabidopsis*. The sequences (*topoisomerase* from pea and *acetolactate synthase* from *Arabidopsis*) are available in the EMBL database at accession No. Y14558 and X51514. These genes were cloned at the NdeI-BglII site and the NcoI-XbaI site.

As such, the actual portions used may not need to be defined since any region out of these genes can be used. The only condition to be satisfied is that the sequence chosen must possess the properties described in claims 10 and 11. Any sequence not possessing these properties, if chosen will not function as an effective insulator.

Portions of the said sequences are selected, mobilized into suitable transformation vector (as described in the application) using convenient restriction sites, the selection of which is also well known to any person skilled in the art and used as insulators.

The length of the insulator sequence described in the present invention is about 5kb of which the contribution from

partial coding sequences of *topoisomerase I* and *acetolactate synthase* gene is about 4.7kb. The final length of about 5 kb is achieved by the incorporation of vector sequences containing various restriction sites that are normally introduced during various intermediate sub-cloning procedures.

As to the "recommendation" issue raised in paragraph 4, page 4 of Office Action, Applicants have only suggested that use of a sequence having certain properties will function as an effective insulator. If a sequence inherently possesses these qualities, it can be used straightaway as insulator. The limitations prescribed are to ensure that the sequence chosen is devoid of transcriptional or other regulatory elements. Besides, the specification is to be read as a whole; parts thereof are not to be selected and dissected for adverse interpretation.

Finally, on page 5, paragraph 2 of the Office Action, the Examiner indicates that the lethal genes are expressed in the tapetum and therefore claim 1 must also be limited to "tapetum." Applicants respectfully disagree. While it is true that the lethal genes selected in this invention are expressed in the tapetum, the teachings of the invention could very well be applied to lethal genes that may be expressed in other regions. In any event, Applicants add claim 32, which recites that the specific tissue(s) is tapetum.

For all of the above reasons, Applicants respectfully submit that the instant claims are enabled by the specification.

Withdrawal of the instant rejection is therefore respectfully requested.

Written Description

The Examiner also rejects claims 1-25 and 27-31 under 35 U.S.C. § 112, first paragraph, for allegedly not being described in the specification. Claims 12, 21, 22, 24, and 25 are canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

The Examiner maintains her position that the insulator sequences are not described in the specification. In the Reply filed on April 10, 2002, Applicants argued that the insulator DNA and the vector as well as each component thereof are well published and known to any person skilled in the art. In response, the Examiner contends that other nucleic acids that can be used as insulators have not been described in the specification. Applicants respectfully disagree.

First of all, the *topoisomerase* I and *acetolactate synthase* genes are merely representative examples. Other coding sequences that may also be used as insulators, such sequences possess the properties set out in claims 10 and 11.

The vector, the sequences of the *bar* and the *barnase* gene, CaMV35S and TA29 promoters, *topoisomerase* and *acetolactate*

synthase genes are all in the public domain and easily accessible to any person skilled in the art. In the Reply filed on April 10, 2002, Applicants set out the publications wherein these components can be found. Therefore, the entire insulator sequence, the vector and each and every component of the insulator construct is published and well known to a person skilled in the art.

Since each of the components as well as the plasmid are publicly available and the method of making the construct is also adequately described in the specification, a skilled person will not have any difficulty in making the construct. In the light of these facts, a deposit is simply not necessary.

For all of the above reasons, Applicants respectfully submit that the instant claims are adequately described in the specification. Withdrawal of the instant rejection is therefore respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner rejects claims 1-25 and 27-31 under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Claims 12, 21, 22, 24, and 25 are canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

Claims 3, 5, and 14

The Examiner asserts that claims 3, 5, and 14 are not in proper Markush format. In response to the Examiner's remarks, Applicants amend the claims into proper Markush format. Thus, the instant rejection is overcome.

Claim 27

The Examiner asserts that claim 27 lacks antecedent basis for the recitation of "the marker gene" in line 3. Applicants respectfully disagree with the Examiner's statement since claim 27 depends from claim 16, which recites "marker gene" in section viii). Further, Applicants amend the recitation of "marker DNA" in section b) of claim 16 to "marker gene," in order to maintain consistency throughout the claims.

Claim 10

The Examiner states that claim 10 lacks antecedent basis for the recitation of "the insulator sequence of about 5kb." In response to the Examiner's remarks, Applicants amend the phrase to, "the insulator sequence is about 5kb in length and…." Thus, the instant rejection is overcome.

Claims 11 and 31

The Examiner asserts that claims 11 and 31 are indefinite for the recitation of "functional…components," which the Examiner asserts is unclear. Applicants delete "functional

components," so that the claims read "…regulatory components or possess any enhancer elements or sequences…."

The Examiner asserts that claims 11 and 31 lack antecedent basis for the recitation of "the host genome." In response to the Examiner's remarks, Applicants amend the phrase to "a host genome."

Further, the Examiner states that claims 11 and 13 are written in a manner that is confusing. The Examiner recommends amending the claims to recite each section with "the insulator sequence…." In response to the Examiner's remarks, Applicants amend claims 11 and 13 accordingly.

Finally, the Examiner states that claims 11 and 13 are indefinite for the recitation of "does not bear strict homology." The Examiner asserts that the level of homology is unclear. Applicants respectfully disagree. As to the issue of "strict homology", the use of the word "strict" in paragraph 4, page 5 of the specification is in accordance with the definition in a dictionary of the English language and would hence stand for "exact or precise, perfect, absolute, rigidly maintained." In the context of the present invention, it is equivalent to 100%.

It is also important to note that in an earlier study by Matzke et al. (copy attached hereto)[1], the authors state that the susceptibility of plant genes to undergo homology-based silencing

[1] Marjori Matzke, Antonius J.M. Matzke and Ortrun Mittelstein Scheid, 1994.

is influenced by the length of uninterrupted homology. In another report (copy attached hereto), it is advocated that one of the mechanisms by which multigene families escape homology-based gene silencing is by decreasing the length of sequence homology[2].

Therefore, it can be concluded that more than the extent of homology between two gene sequences taken in entirety, the actual region of 100% homology (which may represent only a portion of the gene(s)) may be more critical in inducing gene silencing. It is therefore not possible to provide a more precise and independent definition of percent homology alone that would be universally effective in countering homology-based gene silencing and this necessitates the use of more generic terminologies for the same. For example, Niebel et al. (copy attached hereto)[3] state the following: "Similarly Angenent et al. (1993, 1994) reported that the petunia homeotic genes *fbp1* and *fbp2* can only suppress their homologs and other *very* homologous genes while other members with *about* 30% sequence divergence are not affected." Copies of Angenent et al. 1993 and 1994 are attached hereto.[4,5]

"Inactivation of Repeated Genes - DNA-DNA Interaction?" *In* Homologous Recombination and Gene Silencing in Plants-, ed. Jerzy Paszkowski, pp 271-307.

[2] Marjori A. Matzke and Antonius J.M. Matzke, 1995. "How and Why do Plants Inactivate Homologous (Trans)genes?- Plant Physiol. 107:679-685.

[3] F. De Carvalho Niebel, P. Frendo, D. Inze, M. Cornelissen and M. Van Montagu, 1995. "Co-suppression of β-1,3-glucanase genes in *Nicotiana tabacum*" *In* Gene Silencing in higher plants and related phenomena in other eukaryotes- ed. P.Meyer, pp 91-103.

[4] Angenent GC, Franken J, Busscher M, Colombo L, van Tunen AJ (1993) Petal and stamen formation in petunia is regulated by the homeotic gene fbp1. Plant J. 4: 101-112.

[5] Angenent GC, Franken J, Busscher M, Weiss D, van Tunen AJ (1994) Co-suppression of the petunia homeotic gene fbp2 affects the identity of the

Therefore, Applicants emphasize that the "percent homology" referred in the instant application is for a defined length of DNA sequence but could be lesser when entire gene sequences are taken into account for determining their suitability as Insulators.

Claim 16

The Examiner asserts that it is not clear in claim 16 whether the step of generating male sterile lines occurs. In response to the Examiner's remarks, Applicants amend claim 16 to add the steps of transferring the marker gene containing T1 progeny to the field, and identifying the male sterile phenotype among all T1 progeny.

Applicants have also amended the preamble of claim to add *"Brassica juncea."*

Claim 27

The Examiner states that it is unclear whether the step of claim 27 would occur in the base claim 16. Again, Applicants amend claim 16 to add the steps of transferring the marker gene containing T1 progeny to the field, and identifying the male sterile phenotype among all T1 progeny.

Claims 16-25 and 27-28

Finally, the Examiner asserts that claims 16-25 and 27-28 are

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generative meristem. Plant J. 5: 33-34.

incomplete for omitting essential steps. Again, Applicants amend claim 16 to add the steps of transferring the marker gene containing T1 progeny to the field, and identifying the male sterile phenotype among all T1 progeny.

Applicants respectfully submit that the claims, as amended, overcome the rejections brought up by the Examiner. Withdrawal of the rejection under 35 U.S.C. § 112, second paragraph is therefore respectfully requested.

Rejection under 35 U.S.C. § 102

Williams '433

The Examiner maintains the rejection of claims 1-4, 7-9, 13-14, 16-17, 19-20, 24, and 27-28 under 35 U.S.C. § 102(e) for allegedly being anticipated by Williams '433 (USP 5,977,433). Claim 24 is canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

In order to further patentably distinguish the instant claims from Williams '433, Applicants amend base claims 1 and 16 to place a length limitation on the size of the insulator sequence of about 5 kb (as already recited in claim 10). Applicants note that size of the insulator construct varies depending on the selection of

the insulator sequence and other components. However, in any case, the size of the construct does not matter as much as the size of the insulator sequence, which is provided as about 5kb in the amended claims.

Applicants respectfully submit that Williams '433 fails to anticipate the present invention. Specifically, Williams '433 fails to disclose an insulator sequence of about 5 kb in length. Withdrawal of the instant rejection is therefore respectfully requested.

Chang '042

The Examiner also maintains the rejection of claims 1-4, 7-9, and 13-14 under 35 U.S.C. § 102(b) for allegedly being anticipated by Chang '042 (USP 5,610,042). Applicants respectfully traverse. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

In order to further patentably distinguish the instant claims from Chang '042, Applicants amend base claim 1 to place a length limitation on the size of the insulator sequence of about 5 kb (as already recited in claim 10). Applicants respectfully submit that Chang '042 fails to anticipate the amended claims since the sequence disclosed by Chang '042 is only 23 nucleotides long.

Nevertheless, Applicant wish to point out that in paragraph 15 of the Office Action, the Examiner is confusing the size of the construct with the size of the insulator. As noted in the

specification, the insulator sequence functions in the absence of any inhibitor or protein in the background.

For the above reasons, Applicants respectfully submit that Chang '042 fails to anticipate the present invention. Withdrawal of the instant rejection is therefore respectfully requested.

Rejection under 35 U.S.C. § 103

The Examiner maintains the rejection of claims 1-9, 13-14, 16-21, 24-25, and 27-29 under 35 U.S.C. § 103(a) for allegedly being unpatentable over Williams '433 (*supra*) in view of Mariani '041 (USP 5,689,041), as well as the rejection of claims 15 and 22-23 under 35 U.S.C. § 103(a) for allegedly being unpatentable over Williams '433 (*supra*) in view of Mariani '041 (supra) and further in view of Mathews et al (*Plant Science* 72:245 (1990)). Claims 12, 21, 22, 24, and 25 are canceled, thus rendering rejection thereof moot. Applicants respectfully traverse the rejection applied to the pending claims. Reconsideration of the claims and withdrawal of the instant rejection are respectfully requested.

As presented in the Reply filed on April 10, 2002, the hypothetical combination of Williams '433, Mariani '041, and optionally Mathews et al to produce male-sterile plants using the *barnase* gene would work only in situations where the inhibitor protein of *barnase* (barstar) is expressed in the

background.

In order to further patentably distinguish the present invention from the cited references, Applicants amend claim 1 to recite that the insulator sequence functions in the absence of any inhibitor or protein in the background. This limitation clearly distinguishes the present invention from the cited references.

Unlike the method disclosed by Williams '433, Mariani '041, and optionally Mathews et al, the inventive method does not require the presence of inhibitor protein which may vary from one lethal gene to another. Further, the method described in the claims of the present application require obtaining male sterile plant with normal vegetative morphology and normal female fertility at high frequency, backcrossing, etc. (steps (iv) to (ix) of claim 16 which are not described by the prior art suggested by the Examiner.

Therefore, contrary to the position taken by the Examiner, the cited references do not render the invention obvious. Withdrawal of the instant rejection is requested.

Conclusion

In view of the above comments and/or amendments, Applicants respectfully submit that the claims are in condition for allowance. A Notice to such effect is earnestly solicited.

If the Examiner has any questions concerning this

application, she is requested to contact Kristi L. Rupert, Ph.D. (45,702) at (703) 205-8000 in the Washington, D.C. area.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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RCS/KLR:gml
Attachment

Attachments: Version With Markings To Show Changes Made
Combined Declaration and Power of Attorney

Marjori Matzke, Antonius J.M. Matzke and Ortrun Mittelstein Scheid, 1994. "Inactivation of Repeated Genes - DNA-DNA Interaction?" *In* Homologous Recombination and Gene Silencing in Plants-, ed. Jerzy Paszkowski, pp 271-307.

Marjori A. Matzke and Antonius J.M. Matzke, 1995. "How and Why do Plants Inactivate Homologous (Trans)genes?- Plant Physiol. 107:679-685.

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Angenent GC, Franken J, Busscher M, Weiss D, van Tunen AJ (1994) Co-suppression of the petunia

homeotic gene fbp2 affects the identity of the generative meristem. Plant J. 5: 33-34.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

The claims have been amended as follows:

1. (Twice Amended) An insulator construct for controlling leaky expression of a lethal gene from enhancing functions of a strong constitutive promoter present in the said insulator construct following integration into the genome of a plant, said insulator construct comprising:

i) first transcription unit comprising a lethal gene under transcriptional control of a tissue specific promoter for targeted expression in specific tissue(s) and fused to a suitable transcription termination signal, comprising a polyadenylation signal,

ii) second transcription unit comprising a selectable marker gene under transcriptional control of a strong constitutive promoter and fused to a suitable transcription termination signal, comprising a polyadenylation signal, and

iii) an insulator sequence <u>which is about 5kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements</u> placed between the first and second transcription units so as to isolate the first transcription unit from enhancing influences of the constitutively expressing promoter in the second transcription unit,

<u>wherein the insulator sequence functions in the absence of</u>

any inhibitor or protein in the background.

3. (Twice Amended) The construct as claimed in claim 1 wherein the lethal gene is selected from the group consisting of [comprising] *barnase* gene, *RnaseTI* gene, *binase* gene, *rolB* gene, *rolC* gene and diphtheria toxin A gene.

4. (Twice Amended) The construct as claimed in claim 1 wherein the lethal gene is [*is*] *barnase* gene.

5. (Amended) A construct as claimed in claim 1 wherein the tissue specific promoter of first transcription unit is selected from the group consisting of [comprising] TA29, A9, A3, tap1, bcp1, and napin.

10. (Twice Amended) The construct as claimed in claim 1 wherein the insulator sequence is [of] about 5kb in length and comprises coding sequences of *topoisomerase* gene from pea and *acetolactate synthase* gene from *Arabidopsis*.

11. (Twice Amended) The construct as claimed in claim 10 wherein the insulator sequence has the following properties:

(a) the insulator sequence does not encode any [functional or] regulatory components or possess any [regulatory or]

enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content [of the sequence] which is in consonance with transcriptionally active regions of a [the] host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing.

14. (Amended) The plant as claimed in claim 13 which is selected from the group consisting of a dicotyledonous [or] and a monocotyledonous plant [plants].

16. (Twice Amended) A method to obtain male-sterile plants in *Brassica juncea*, said method comprising the steps of:

i) transforming the nuclear genome of plant cells with a foreign DNA comprising:

a) a first transcription unit comprising a lethal gene under transcriptional control of a tissue specific promoter for targeted expression in specific tissue(s) and fused to a suitable transcription termination signal,

comprising a polyadenylation signal,

b) a second transcriptional unit comprising a selectable marker gene [DNA] under transcriptional control of a strong constitutive promoter and fused to a suitable transcription termination signal, comprising[,] a polyadenylation signal, and

c) an insulator sequence which is about 5kb in length, and which does not comprise transcriptional or other regulatory or enhancer elements placed between the first and second transcription units, so as to isolate the first transcription unit from enhancing influences of the constitutively expressing promoter in the second transcription unit;

ii) regenerating plants from said transformed plant cells,

iii) identifying male sterile transgenic plants by the absence of pollen production and by their failure to set seed on selfing,

iv) obtaining, at a high frequency, male sterile plants with normal vegetative morphology and normal female fertility,

v) identifying single copy male sterile lines by Southern hybridization,

vi) back-crossing male sterile plants with untransformed parent to obtain T1 seeds,

vii) obtaining male sterile plants with normal T1 seed germination frequencies,

viii) obtaining normal segregation ratio of the marker gene among T1 progeny of single copy male sterile plants identified, [and]

ix) transferring the marker gene containing T1 progeny to the field, and

x) identifying the [obtaining stable transfer of] male sterile phenotype among all T1 progeny [plants] exhibiting marker resistance.

23. (Twice Amended) A method as claimed in claim 16 wherein male sterile lines in *Brassica* [*Brassican*] *juncea* are generated by *Agrobacterium*-mediated transformation using disarmed Ti plasmid.

26. (Twice Amended) A method as claimed in claim 16 wherein T1 seeds [of claim 25] are tested for their viability as evidenced by their ability to germinate on non-selective media.

27. (Twice Amended) A method as claimed in claim 16 wherein germinated T1 seedlings obtained from backcrossed seeds [were] are tested for segregation of the marker gene by transferring them [on] onto selective media.

30. (Amended) A method as claimed in claim 16, wherein the

insulator sequence comprises coding sequences of *topoisomerase* gene from pea and [acid] *acetolactate synthase* gene from *Arabidopsis*.

31. (Amended) A method as claimed in claim 16, wherein the insulator sequence [Sequence] comprises the following properties:

(a) the insulator sequence does not encode any [functional or] regulatory components or possess any [regulatory or] enhancer elements or sequences that may influence the expression of neighboring genes;

(b) the insulator sequence has a GC content [of the sequence] which is in consonance with transcriptionally active regions of a [the] host genome;

(c) the insulator sequence does not produce any functional RNA or protein; and

(d) the insulator sequence does not bear strict homology with any component of the host genome in order to avoid induction of homology dependent gene silencing.

Claims 12, 21, 22, 24, and 25 are canceled.

Claim 32 is added.